Neuroscience Letters, 158 (1993) 209–212

© 1993 Elsevier Scientific Publishers Ireland Ltd. All rights reserved 0304-3940/93/$ 06.00

NSL 09728

Deficits in working but not reference memory in adult rats in which limbic seizures had been induced before weaning: implications for early brain injuries

Maria Kostakos, M.A. Persinger and Oksana Peredery

Behavioral Neuroscience Laboratory, Neuroscience Program, Laurentian University, Sudbury, Ont. (Canada)

(Received 24 March 1993; Revised version received 7 May 1993; Accepted 7 May 1993)

Key words: Radial arm maze; Rat; Limbic seizure; Lithium; Ontogeny; Reference memory vs. working memory

Male adult rats that displayed limbic seizures between postnatal days 18 and 21 after a single s.c. injection of Li followed 4 h later by a muscarinic agent were trained in a radial arm maze; they were compared with rats that had received the Li-pilocarpine (but had not displayed overt seizures) and to nonhandled controls. Only the rats that had displayed the (preweaned) seizures displayed significant impairment for working memory but not for reference memory. Light microscopy demonstrated histological evidence of earlier damage only within select thalamic structures that are directly associated with the amygdaloid–hippocampal complex. The results are compatible with the hypothesis that early seizures during the time of CA1 hippocampal maturation can produce long-term changes in the efficacy of short-term memory.

Seizure-induced brain damage in adult rats, induced by a single systemic injection of Li (3 mEquiv/kg) followed 4 h later by 30 mg/kg of pilocarpine [12] is associated with serious deficits in radial arm maze performance [3, 11]. Subsequent experiments have shown that both reference and working memory are significantly disrupted. The behavioral patterns are remarkably similar to those induced by experimental lesions of the mediodorsal nucleus and are highly correlated with measures of necrosis for this region but not the hippocampal formation.

The multifocal brain damage that occurs within the adult rat brain following the Li-pilocarpine injections are substantial. We reasoned that induction of these seizures within the developing brain, especially during the preweaning (21 days) period when the CA1 portion of the hippocampal formation is undergoing maximum synaptogenesis [2, 17] would preferentially influence this area. The correlative disruption in synaptogenesis should encourage a permanent dissociation between working memory and reference memory. Whereas other (thalamic) structures would be expected to recover from or to compensate for the adverse cellular correlates of the seizure, the cytoarchitectural sequelae of the early seizure should induce synaptic organizations (within the amygdaloid–hippocampal complex) that encourage electrical seizures throughout life. If this hypothesis is correct, then such rats should display severe deficits in working but not reference memory in a manner similar to the pattern reported [5, 6].

In two separate blocks of experiments, 26 140–150-day-old Wistar albino male rats served as subjects. Between the ages of 17 and 20 days, eight of the rats had received a treatment that induced limbic seizures; another nine rats had received the same treatment but no seizures were observed. Nine other rats served as nonhandled controls. All rats were bred within the colony; each litter contained nine pups. A total of nine litters were recruited for this study; six rats from each litter received 3 mEquiv/kg of Li chloride followed 4 h later by 30 mg/kg of pilocarpine; both injections were given s.c. If the rat displayed forepaw clonus and clear indications of limbic seizures, it was marked with a right ear punch. If the rat, injected with the same two chemicals, did not display the seizures, it was marked with a left ear punch. The other three rats (controls) per litter were not touched. After weaning (21 days) rats were housed three per cage with food and water ad libitum.

For maze training, one rat from each of the three conditions (Li-pilocarpine:seizure verified; Li-pilocarpine:

Correspondence: M.A. Persinger, Behavioral Neuroscience Laboratory, Neuroscience Program, Laurentian University, Sudbury, Ontario P3E 2C6, Canada.

no seizure and control) was selected from each litter. The reduced size of the first group occurred because of the greater mortality between the seizure and training day. Training began when the rats were 140 days of age, after reduction to 85% body wt during an 1-wk period. Each rat was shaped to gradually enter each of the eight arms of the radial arm maze over a 3-day period. Two fruit loops per arm were used as rewards.

During the 7 test days, only four of the arms contained the rewards. For about half the numbers of rats, the odd-numbered arms were baited consistently while for the other rats the even-numbered arms were baited consistently. Animals were tested daily until all four baited arms had been entered or a maximum of 15 min had elapsed. If an arm had not been entered by this time, the remaining unentered arms were listed as reference memory errors. Working memory errors were defined as the numbers of times a rat reentered an arm in which it had entered previously (baited or nonbaited) during the test session; the session ended when all arms at been entered at least once. The experimenter (first author) who trained and tested the rats was not familiar with their treatment conditions.

After decapitation, the brains from six of the rats (three in which overt seizures had been induced and three that received the drugs but in which no overt seizures were induced) were removed; the cerebrums were fixed in ethanol-formalin-acetic acid and processed [10]. Every 20th (200 μm), 10-μm section between the posterior commissure and the anterior commissure was stained with toluidine blue O. The entire surface of each section was examined at 100 and 400× light microscopy; all structures indicated by Paxinos and Watson [9] were evaluated for: (a) neuronal dropout, (b) gliosis or (c) the accumulation of crystalline forms of cellular debris by an experienced histomorphologist [7,11].

Two-way ANOVA with one level repeated (sessions) and nonrepeated (treatment groups) were completed for the working and reference memory errors separately; a three-way ANOVA with two levels repeated (type of memory; sessions) was also conducted. All analyses were completed on SPSSX software using a Vax 4000 computer.

The mean numbers of short- (working) and long-term (reference) errors displayed by the three groups over the seven sessions are shown in Fig. 1 . The mean and S.D. values (the latter between parentheses) for the numbers of working memory errors per session for the control, early drug control (Li-pilocarpine but no observed seizures) and early seizured groups were 3.6 (1.7), 2.8 (1.2) and 5.9 (1.2), respectively; post hoc analyses ($P < 0.05$) indicated that the significant [$F_{2,23} = 10.89$; $P < 0.01$] differences between the groups was due to the greater numbers of errors that were displayed by the seizured group compared with the other two groups who did not differ significantly from each other. The treatment history explained 49% of the variance in short-term memory errors. The mean and S.D. values for the numbers of reference memory errors per day for the three groups for the entire experiment were 3.6 (1.7), 2.4 (1.7) and 3.8 (1.7), respectively; the group differences were not significant statistically [$F_{2,23} = 1.65$, $P > 0.05$].

Three-way ANOVA with two levels repeated (reference vs. working memory; sessions) demonstrated the significant group differences [$F_{2,23} = 7.30$, $P < 0.01$]; although the significant difference between types of memory (more working errors) was significant [$F_{1,23} = 4.50$, $P < 0.05$], the visually apparent source of this effect, the group by memory interaction was not [$F_{2,23} = 2.64$, $0.05 < P < 0.10$]. Although the learning curve (session difference) was statistically significant [$F_{6,138} = 4.82$, $P < 0.001$], the group by sessions [$F_{12,138} = 0.60$, $P > 0.05$], type of memory by sessions [$F_{6,138} = 0.98$] and group by memory by sessions [$F_{12,138} = 1.07$] interactions were not.

Because of the expected discrepancy between the variance in errors displayed over test sessions, square-root transformations were completed for the numbers of reference and working errors. Subsequent analyses reaffirmed the group differences [$F = 7.46$]; none of the other effects were statistically significant. To discern the maximum strength of the effect, two-way ANOVAs with one level (sessions) repeated were completed separately for reference and working memory. For the working memory, both the group differences [$F_{2,23} = 10.81$, $P < 0.001$] and the session differences [$F_{6,138} = 5.17$, $P < 0.001$] were significant; the interaction was not. Analyses of the reference memory errors only did not discern a group difference [$F = 1.65$] or a group by sessions interaction [$F = 0.75$], although the session differences were significant [$F_{6,138} = 3.30$, $P < 0.01$].

Light microscopy revealed surprisingly little discriminable necrosis within the telencephalon or diencephalon of the rats in which seizures had been induced before weaning. There was no evidence of damage within the substantia nigra reticulata, hippocampal formation, entorhinal/piriform cortices, neocortices or amygdaloid complex (these structures were invariably damaged in rats that were killed a comparable time after the inductions of seizures by the same dosage of Li-pilocarpine during adulthood; [7, 11, 12]). Focal damage, as defined by gliosis and accumulation of crystallized forms of cell debris [7], within at least 50% (measured quantitatively) of the area delineated by Paxinos and Watson as a distinct structure, was found only in the thalamus. The anomalies were discretely located within the medial dor-

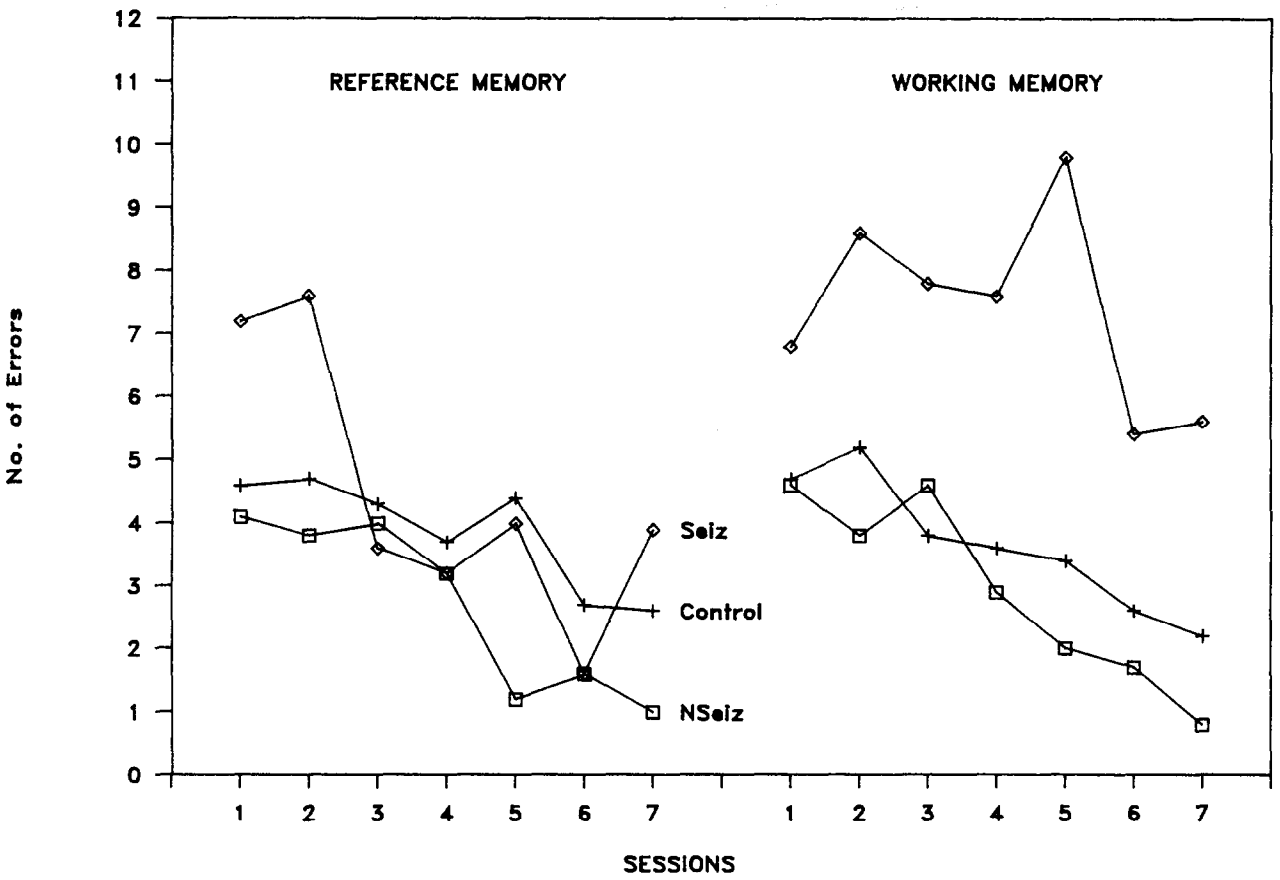

Fig. 1. Mean numbers of errors per daily session within radial arm maze for adult rats: (1) in which seizures had been induced by preweaning s.c. injections of Li and pilocarpine (Seiz); (2) in which no overt seizures had occurred despite injections (NSeiz); or (3) that served as nonhandled controls. Reference and working memories are indicated.

sal group (principle MD and lateral MD), the posterior group (posterior complex and triangularis), the lateral complex (lateral posterior and laterodorsal nuclei), the suprageniculate nucleus and dorsal component of the medial geniculate nucleus and either the nucleus reuniens or nucleus gelatinosus of the midline group; granules of old cell debris were evident in portions of the ventroposterior lateral and ventromedial complex.

These results support the ideas by Green and Stanton [2] that dissociations between reference and working memory occur across ontogeny especially during the period when Schaeffer collaterals synapse with CA1 hippocampal neurons [17]. Induced disruptions in normal neuronal activity due to seizure activity during a time when the neurosubstrate of working memory is emerging should produce long-term deficits without affecting the capacity for reference memory. In the present study, induction of limbic seizures by a single systemic injection of Li and pilocarpine between postnatal days 18 and 21 resulted in differential discrepancies between these two forms of memory. As adults, rats in which these seizures had been induced displayed persistent and more significant working memory errors compared with control rats while reference memory was essentially unaffected.

However, unlike the massive neuronal loss within the CA1 region of the hippocampus, entorhinal cortices, piriform cortices and most of the amygdaloid complex that occurs in adult rats in which Li-pilocarpine seizures have been induced [11, 12] the rats in the present study did not display extensive brain damage. Instead, areas of high density gliosis or highly localized (50–100 μm diameter) accumulation of crystalline cell debris were found within the mediodorsal, posterior, lateral, medial geniculate and midline groups. The components of these complexes that were most damaged share inputs into the CA1 region of the hippocampus, its primary afferent source (the entorhinal cortices) and the basolateral amygdaloid complex [1, 4, 8, 15, 16].

In light of these connections and the observed histomorphology, the most compelling and parsimonious explanation for the present results would involve kindling of the amygdaloid–hippocampal complex by excessive phasic discharges from these thalamic regions during the weeks that followed the induction of the seizures. That

these discharges were extreme and sufficient to produce neuronal death in the present study can be inferred by the presence of gliosis and the accumulation of cell fragments. They were still conspicuous by light microscopy > 100 days after the induction of the seizure. Because kindling within the hippocampal–amygdaloid structures is primarily a process that involves synaptic reorganization, structural changes should not be (and were not) discernable at the 100 and 400× levels of inspection.

The importance of the developmental stage of the organism at the time of limbic seizure induction is also emphasized in this study. The results reinforce the clinical suspicion that working (short-term) memory is more vulnerable than long-term memory to smaller amounts of brain damage and/or subtle synaptic reorganization of the hippocampal–amygdaloid complex. In fact, the dissociation between working and reference memory and the specificity of thalamic damage in adult rats that sustained early neuronal loss suggests a model for both the understanding and treatment of attention deficits in human beings. Early episodes of hypoxia and (presumably) diffuse neuronal loss within the cerebral cortices are often associated with later development of attention deficits. Because cortical neuronal loss is ultimately associated with thalamic necrosis [13], electrical bursts to the hippocampal–amygdaloid complex during successive episodes of excitotoxic deterioration of afferent thalamic regions could drive a kindling process.

Commensurate with the results of our study, children who display moderate to extreme attention deficits have greater difficulty with short-term (hippocampal) rather than long-term memory. The involvement of the amygdala would increase the likelihood of emotional (particularly aggression, episodes of fear), complications that could be manifested both as either hyperactivity or as 'behavioral' problems that include orality and 'hypersexuality'. The session-dependent dissociation between short- and long-term memories (which was impaired during the first two sessions) suggests that: (1) information is ultimately consolidated in long-term memory although more repetitions are required; (2) normal working memory is a sufficient but not a necessary condition for reference memory; and (3) the amount of long-term memory (e.g., Information) can be normal despite impaired short-term memory (e.g., Digit Span).

We thank Yves Bureau for technical assistance.

1 Cornwall, J. and Phillipson, O.T., Afferent projections to the dorsal thalamus of the rat as shown by retrograde lectin transport-I. the mediodorsal nucleus, Neuroscience, 24 (1988) 1035–1049.
2 Green, R.J. and Stanton, M.E., Differential ontogeny of working memory and reference memory in the rat, Behav. Neurosci., 103 (1988) 98–105.
3 Harrigan, T., Peredery, O. and Persinger, M.A., Radial maze learning deficits and mediodorsal thalamic damage in context of multifocal, seizure-induced brain lesions, Behav. Neurosci., 105 (1991) 482–486.
4 Herkenham, M., The connections of the nucleus reuniens thalami: evidence for a direct thalamo–hippocampal pathway in the rat, J. Comp. Neurol., 177 (1978) 589–610.
5 Knowlton, B., McGowan, M. and Olton, D.S., Hippocampal stimulation disrupts spatial working memory even 8 hr after acquisition, Behav. Neural Biol., 44 (1985) 325–337.
6 Knowlton, B.J., Shapiro, M.L. and Olton, D.S., Hippocampal seizures disrupt working memory performance but not reference memory acquisition, Behav. Neurosci., 103 (1989) 1114–1147.
7 Lafrenière, G., Peredery, O. and Persinger, M.A., Progressive accumulation of large aggregates of Ca^{2+}-containing polysaccharides and basophilic debris within specific nuclei of the thalamus after lithium/pilocarpine-induced seizures, Brain Res. Bull., 28 (1992) 825–830.
8 LeDoux, J.E., Farb, C. and Ruggiero, D.A., Topographic organization of neurons in the acoustic thalamus that project to the amygdala, J. Neurosci., 10 (1990) 1043–1054.
9 Paxinos, G. and Watson, C., The Rat Brain in Stereotaxic Coordinates, 2nd edn., Academic Press, Orlando, FL, 1986.
10 Persinger, M.A., Degranulation of brain mast cells in young albino rats, Behav. Neural Biol., 39 (1983) 299–306.
11 Persinger, M.A., Bureau, Y., Kostakos, M. and Peredery, O., Behavioral deficits in rats with insidious brain damage induced by seizures following single injections of lithium and pilocarpine, Physiol. Behav., 53 (1993) 849–866.
12 Persinger, M.A., Makarec, K. and Bradley, J.-C., Characteristics of limbic seizures evoked by peripheral injections of lithium and pilocarpine, Physiol. Behav., 44 (1987) 27–37.
13 Ross, D.T. and Ebner, F.F., Thalamic retrograde degeneration following cortical injury: an excitotoxic process? Neuroscience, 35 (1990) 525–550.
14 Stokes, K.A. and Best, P.J., Mediodorsal thalamic lesions impair radial maze performance in the rat, Behav. Neurosci., 102 (1988) 294–300.
15 Su, H.-S. and Bentivoglio, M., Thalamic midline cell populations projecting to the nucleus accumbens, amygdala, and hippocampus in the rat, J. Comp. Neurol., 297 (1990) 582–593.
16 van Vulpen, E.H.S. and Verwer, R.W.H., Organization of projections from the mediodorsal nucleus of the thalamus to the basolateral complex of the amygdala in the rat, Brain Res., 500 (1989) 389–394.
17 Wasterlain, C.G., Hattori, H., Yang, C., Schwartz, P.H., Fujikawa, D.G., Morin, A.M. and Dwyer, B.E., Selective vulnerability of neuronal subpopulations during ontogeny reflects discrete molecular events associated with normal brain development. In C.G. Wasterlain and P. Vert (Eds.), Neonatal Seizures, Raven Press, New York, 1989, pp. 69–81.